UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA VEGETAL

Isoeugenol monooxygenase characterization and whole-cell bioconversion systems towards vanillin production from plant-derived precursor isoeugenol

Francisco Quintas Nunes

Mestrado em Microbiologia Aplicada

Versão Pública

Dissertação orientada por:
Prof. Doutora Lígia O. Martins
Prof. Doutora Manuela Carolino

2019

O trabalho apresentado nesta Dissertação de Mestrado foi realizado no Instituto de Tecnologia Química e Biológica (ITQB) sob a orientação direta da Professora Doutora Lígia O. Martins

A Professora Doutora Maria Manuela Spratley Saraiva de Lemos Carolino foi a orientadora interna, designada no âmbito do Mestrado em Microbiologia Aplicada da Faculdade de Ciências da Universidade de Lisboa

Acknowledgments

This work would not be possible if I did not have some people in my life. For that, I would like to say thank you.

First, I would like to thank my supervisor Lígia, for giving me the opportunity to be a part of this mind-blowing and solution driven European project. For supporting and teaching me science skills throughout this whole year. For believing in me and my abilities, especially when I did not. A very big thank you.

To Vânia for always having my back in the lab. For the support and teachings.

Thank you to Diogo, Diana, Ana, André, Vera, Magdalena, Magali, Maria, Elisa, Mariana and Catarina for every moment spent with me, in and out of the lab, for keeping me company and cheering me up, each in its own unique way. For the laughs, knowledge and companionship we shared.

To the research facilities technicians at ITQB, Isabel, Teresa, Cristina, Paula, Helena and João, a big thank you for the help, support and tips provided during the lab work.

To Professor Manuela for chaperoning me since the bachelor's degree, guiding me through these academic experiences and teaching me about science.

To all my family, parents, sister and friends for always being there for me cheering every step of the way.

Thank you so much!

Abstract

This work is a response to the crescent interest by the biorefinery industries that drive research towards more cost-competitive and environmentally sustainable processes. New ways to value materials that would once be considered wasteful or valueless are researched. Lignin is one of such cases, a complex biopolymer that is considered by the paper and ethanol producing industries as waste. Most importantly, lignin is the only large-volume renewable feedstock that contains aromatic compounds, making it an attractive candidate for research efforts towards its valorization.

In this study, isoeugenol, a compound resultant from lignin reductive catalytic fractionation (RCF) was used as substrate for vanillin production. This bioconversion was achieved resorting to an isoeugenol monooxygenase as biocatalyst. At start, the heterologous production of two isoeugenol monooxygenases, one from *Pseudomonas putida* (PpIEMO) and one from *P. nitroreducens* (PnIEMO) were compared regarding protein solubility and enzyme activity, in 50 mL scale cultures using different *E. coli* strains. The isoeugenol monooxygenase from *P. nitroreducens* was then picked to proceed to scale-up and characterization processes using *E. coli* Tuner Δ*cueO* as host. After production at 1L-scale, PnIEMO was purified by affinity chromatography techniques and biochemically characterized to assess its potential application in biotechnology. The evaluating of its properties and limitations will allow in the future, to proceed to enzyme evolution. Purification of PnIEMO from 1 L of growth medium yielded ≈ 1.8 mg of purified enzyme. The molecular mass determined was of 50.1 kDa. The optimum pH and temperature for this enzyme were pH 9 and 30 °C. Michaelis Menten parameters were calculated and resulted in K_m and K_m/K_{cat} of 0.13 mM and 118 mM^{-1} s^{-1}, respectively. PnIEMO presented low stability towards temperature, with half-life of 7 min at 35 °C and a melting temperature of 32 °C (temperature at which 50 % of the molecules are unfolded), when following tryptophan fluorescence as protein denaturation indicator. This enzyme presented some tolerance towards guanidine hydrochloride, displaying a midpoint of the concentration of 1.3 M (where 50 % of molecules are unfolded). The techniques assessed to improve the yields of soluble enzyme, namely the use of hosts co-expressing chaperones, or to recover active enzyme from the inclusion bodies present in the cell pellets, by unfolding and refolding processes, did not show improvements. Whole-cell assays were performed using free and alginate immobilized *E. coli* cells expressing PnIEMO towards isoeugenol bioconversion to vanillin. Both approaches enabled ≈ 100 % vanillin production in 24 h, although using free whole-cells the reaction was faster than with alginate immobilized whole-cells. Summing up, this enzyme is an excellent candidate for use in biotechnological vanillin production processes, using isoeugenol resultant from lignin RCF.

Keywords: Lignin valorisation, isoeugenol, vanillin, isoeugenol monooxygenase, whole-cell reactions